

Appl. No. 09/147,693
Amdt. Dated Nov. 3, 2003
Reply to Final Office Action of June 3, 2003

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application.

Listing of claims:

1-37. Canceled.

38. (Previously presented) A method for selecting OR or OL operator DNA sequences from lambdoid phages wherein said sequences have a different thermostability compared to a wild-type sequence with regard to binding a repressor, wherein said different thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, comprising

- (a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,
- (b) intentionally subjecting the operator DNA sequence to a mutagenesis, and
- (c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

39. (Currently amended) The method according to claim 38, wherein the lambdoid phages are selected from the group consisting of phage lambda, phage 21, phage 22, phage 82, phage 424, phage

Appl. No. 09/147,693
Amdt. Dated Nov. 3, 2003
Reply to Final Office Action of June 3, 2003

434, phage D326, DLP12, phage gamma, phage HKO22, phage P4, phage Phi80, phage Phi81, and coliphage 186.

40. (Previously presented) The method according to claim 39, wherein said lambdoid phage is phage lambda.

41. (Previously presented) The method according to claim 40, wherein said operator DNA sequence is from the operator regions OR and/or OL of the phage lambda.

42. (Previously presented) The method according to claim 38, wherein said selection gene is an E-lysis gene from phage PhiX174.

43. (Previously presented) The method according to claim 38, wherein the operator DNA sequence is subjected to a site-specific mutagenesis by oligonucleotides or a selection is carried out in a mutator bacterial strain.

44. (Previously presented) The method according to claim 38, wherein the operator DNA sequences are analyzed by determining their ability to bind to a temperature-sensitive cl repressor.

45. (Previously presented) The method according to claim 44, wherein temperature-sensitive lambda cl repressor is cl857.

Appl. No. 09/147,693
Amdt. Dated Nov. 3, 2003
Reply to Final Office Action of June 3, 2003

46-48. Canceled.

49. (Currently amended) An isolated lambda OR operator sequence comprising the sequence shown in SEQ ID NO[[.]]; 2.

50. (Previously presented) A nucleic acid comprising a bacterial expression control sequence containing a OR or OL operator sequence according to claim 46 in operative linkage with a protein-coding sequence.

51. (Previously presented) The nucleic acid according to claim 50, wherein the protein-coding sequence is a suicide gene.

52. (Previously presented) The nucleic acid according to claim 50, wherein the expression control sequence contains a lambda PL or PR promoter.

53. (Previously presented) A vector comprising at least one copy of a nucleic acid according to claim 50.

54. (Previously presented) The vector according to claim 53, wherein said vector is a bacterial chromosomal vector.

Appl. No. 09/147,693
Amdt. Dated Nov. 3, 2003
Reply to Final Office Action of June 3, 2003

55. (Previously presented) The vector according to claim 53, wherein said vector is a bacterial extrachromosomal plasmid.

56. (Previously presented) A bacterial cell transformed with a nucleic acid according to claim 50.

57. (Previously presented) A bacterial cell transformed with a vector according to claim 53.

58. (Previously presented) A bacterial cell according to claim 56, wherein said nucleic acid is integrated into said cell's chromosome.

59. (Previously presented) A bacterial cell according to claim 57, wherein said vector is integrated into said cell's chromosome.

60. (Previously presented) A bacterial cell according to claim 56, further comprising a gene for a cl repressor from lambdoid phages.

61. (Previously presented) A bacterial cell according to claim 57, further comprising a gene for a cl repressor from lambdoid phages.

62. (Previously presented) A bacterial cell according to claim 60, wherein said gene is the lambda cl857 repressor.

Appl. No. 09/147,693
Amdt. Dated Nov. 3, 2003
Reply to Final Office Action of June 3, 2003

63. (Currently amended) ~~A vaccine~~ An immunogenic composition, comprising a live bacterial cell according to claim 56 in combination with pharmaceutically acceptable auxiliary substances, additives or carrier substances.

64. (Currently amended) ~~A vaccine~~ An immunogenic composition, comprising a live bacterial cell according to claim 57 in combination with pharmaceutically acceptable auxiliary substances, additives or carrier substances.

65. (Currently amended) ~~A vaccine~~ An immunogenic composition, comprising a bacterial ghost in combination with pharmaceutically acceptable auxiliary substances, additives and carrier substances in which the bacterial ghost can be obtained by culturing a bacterial cell as claimed in claim 57 at temperatures of 35 - 39°C and subsequently lysing the bacterial cell by increasing the temperature.

66-68. Canceled.

69. (Previously presented) A bacterial cell comprising at least one copy of a nucleic acid, wherein said nucleic acid comprises (a) a first bacterial expression control sequence which contains an OR or OL operator sequence from a lambdoid phage and to which a first cl repressor from lambdoid phages can bind, in operative linkage with a sequence coding for a second repressor wherein the second repressor cannot bind to the first bacterial expression sequence and (b) a second bacterial expression control sequence to which the second repressor can bind in operative linkage with a

Appl. No. 09/147,693
Amdt. Dated Nov. 3, 2003
Reply to Final Office Action of June 3, 2003

suicide gene, wherein said first bacterial expression control sequence is an operator sequence from a lambdoid phage wherein said sequence has a different thermostability compared to a wild-type sequence with regard to binding of a repressor wherein said different thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, and wherein said operator sequence is obtained by a method comprising

- (a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,
- (b) intentionally subjecting the operator DNA sequence to a mutagenesis, and
- (c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

70. (Previously presented) A bacterial cell comprising at least one copy of a nucleic acid, wherein said nucleic acid comprises (a) a first bacterial expression control sequence which contains an OR or OL operator sequence from a lambdoid phage and to which a first cl repressor from lambdoid phages can bind, in operative linkage with a sequence coding for a second repressor wherein the second repressor cannot bind to the first bacterial expression sequence and (b) a second bacterial expression control to which the second repressor can bind in operative linkage with a suicide gene,

Appl. No. 09/147,693
Amdt. Dated Nov. 3, 2003
Reply to Final Office Action of June 3, 2003

further comprising (c) a third bacterial expression control sequence which contains a operator sequence in operative linkage with a suicide gene, wherein said operator sequence is from a lambdoid phage and wherein said operator sequence has a different thermostability compared to a wild-type sequence with regard to binding of a repressor, wherein said different thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, and wherein said operator sequence is obtained by a method comprising

- (a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,
- (b) intentionally subjecting the operator DNA sequence to a mutagenesis, and
- (c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

71. (Currently amended) ~~A vaccine~~ An immunogenic composition, comprising a live bacterial cell according to claim 69 in combination with pharmaceutically acceptable auxiliary substances, additives or carrier substances.

Appl. No. 09/147,693
Amdt. Dated Nov. 3, 2003
Reply to Final Office Action of June 3, 2003

72. (Currently amended) The vaccine immunogenic composition according to claim 71, wherein said vaccine immunogenic composition is a heat-sensitive, a cold-sensitive or both a heat and cold sensitive safe ~~live~~ vaccine immunogenic composition.

73-76. Canceled.

77. (Previously presented) The bacterial cell of claim 69, wherein said bacterial cell further comprises a gene for a first cl repressor.

78. (Previously presented) The bacterial cell of claim 70, wherein said bacterial cell further comprises a gene for a first cl repressor.